REMARKS

Claim Amendments

Claims 34, 35, 38, 39, 42-44 and 50-113 have been canceled in favor of new claims 114-234. For the reasons that follow, Applicants submit that these claims do not constitute new matter and their entry is requested.

Summary of the Claims

The claims have been divided into three sets of claims, each directed to one of the reference RNA sequences, as suggested by Examiner Moran during the telephone interview held on 8 November 2005. These sets of claims are summarized as follows.

Claims 114-151 are directed to processes for quantitation of a target viral RNA sequence in a sample, an amplification reaction mixture (claims 146-147), a reverse transcription reaction mixture (claims 148-149) and a kit (claims 150-151). The process involve the simultaneous amplification of a target viral RNA sequence and reference RNA sequence (claims 114-121 and 130-137) or involve first a simultaneous reverse transcription and then a simultaneous amplification of a target viral RNA sequence and reference RNA sequence (claims 122-129 and 138-145). The reference RNA sequence of claims 114-151 is a reference RNA sequence that consists of the target viral RNA sequence with a multibase insert into a site within the target viral RNA sequence. This reference RNA sequence corresponds to reference RNA sequence (ii) of the previously examined claims.

Claims 152-189 are directed to processes for quantitation of a target viral RNA sequence in a sample, an amplification reaction mixture (claims 184-185), a reverse transcription reaction mixture (claims 186-187) and a kit (claims 188-189). The process involve the simultaneous amplification of a target viral RNA sequence and reference RNA sequence (claims 152-159 and 168-175) or involve first a simultaneous reverse transcription and then a simultaneous amplification of a target viral RNA sequence and reference RNA sequence (claims 160-167 and 176-183). The reference RNA sequence of claims 152-189 is a reference RNA sequence that consists of the target viral RNA sequence with a multibase deletion from a site within the target

viral RNA sequence. This reference RNA sequence corresponds to reference RNA sequence (iii) of the previously examined claims.

Claims 190-234 are directed to processes for quantitation of a target viral RNA sequence in a sample, an amplification reaction mixture (claims 226-228), a reverse transcription reaction mixture (claims 229-231) and a kit (claims 232-234). The process involve the simultaneous amplification of a target viral RNA sequence and reference RNA sequence (claims 190-198 and 208-216) or involve first a simultaneous reverse transcription and then a simultaneous amplification of a target viral RNA sequence and reference RNA sequence (claims 199-207 and 217-225). The reference RNA sequence of claims 190-234 is a reference RNA sequence that comprises a sequence present in the target viral RNA sequence and a sequence that is not present in the target viral RNA sequence. This reference RNA sequence corresponds to reference RNA sequence (iv) of the previously examined claims.

Support for Claim Language

The language "known quantity" is supported by the application at page 7, lines 2-4 and 15-19. The language "multibase insert into a site within the target viral RNA sequence" is supported in the application at page 6, lines 15-25, page 7, lines 9-19, and in original claims 18 and 19. The language "multibase deletion from a site within the target viral RNA sequence" is supported in the application at page 6, lines 15-25. The language "comprises a sequence present in the target viral RNA sequence and a sequence that is not present in the target viral RNA sequence" is supported by the disclosure of a target sequence that includes an insert, such as found in the application at page 6, lines 15-25, page 7, lines 9-19, and in original claims 18 and 19. The language "consists of a linear arrangement of a sequence present in the target viral RNA sequence, a sequence not present in the target viral RNA sequence and a sequence present in the target viral RNA sequence" is supported by the disclosure of a target sequence that includes an insert, such as found in the application at page 6, lines 15-25, page 7, lines 9-19, and in original claims 18 and 19. Adding the reference RNA sequence to the sample is supported in the application at page 7, lines 2-4 and 15-19. Distinguishing the amplified products by size or by probes is supported in the application at page 6, lines 26-29 and page 7, lines 20-27. Measuring

the amounts of amplified products is supported in the application at page 7, lines 1-8 and 15-19 and in original claims 27 and 30. Determining the amount of target viral RNA present in the sample is supported in the application at page 7, lines 1-8 and 15-19 and in original claim 27.

Summary of the Telephone Interview

Applicants would like to thank Examiner Miller and her supervisor, Examiner Moran, for the courtesies extended to the undersigned, the inventor, Dr. John J. Rossi, and a representative of the assignee, Dr. Brian Clark, during the telephone interview on 8 November 2005. During the interview Dr. Rossi presented a brief discussion of the background leading up to the invention and of the invention itself. Draft amended claims were discussed as means to clarify the invention and to distinguish the prior art. The Examiners noted potential interpretation issues of the draft amended claims and the necessity of indicating support in the application for each amendment that may ultimately be made. The Examiners also noted that Section 112 issues could potentially arise and that Applicants should carefully consider the language of their claims. Finally, the Examiners noted that there was a body of prior art by the inventors, including that listed on the Information Disclosure Statement filed on 4 June 2004. The Examiners suggested that Applicants address this prior art in their response in order to expedite the prosecution of the application. Finally, the prior art cited in the previous Office Action was briefly discussed. Applicants pointed out that this prior art did not use a reference sequence that contains target sequence and did not include the addition of a known quantity of a reference sequence to the sample prior to amplification. No agreement was reached with respect to the patentability of the subject matter of the application.

Summary of the Invention

The present invention is directed to a method for the quantitation of target viral RNA in a sample by simultaneously amplifying a target viral RNA sequence and a known quantity of a reference RNA sequence as an internal standard. That is, the target viral RNA sequence, if present, and the reference sequence are simultaneously amplified in the same reaction mixture. The quantity of target viral RNA present in the sample is determined by comparing the amount

of the amplified target viral RNA and the amount of the amplified reference RNA based on the known quantity of reference RNA added as an internal control. The reference RNA sequence may be (a) a reference RNA sequence that consists of the target viral RNA sequence with a multibase insert into a site within the target viral RNA sequence, (b) a reference RNA sequence that consists of the target viral RNA sequence with a multibase deletion from a site within the target viral RNA sequence or (c) a reference RNA sequence that comprises a sequence present in the target viral RNA sequence and a sequence that is not present in the target viral RNA sequence.

Priority

According to the first paragraph of the specification, the present application is a continuation-in-part of three applications, Serial No. 07/355,296, filed 22 May 1989, Serial No. 07/143,045, filed 12 January 1988 and Serial No. 07/148,959, filed 27 January 1988. Thus, the present application claims priority to each of these three applications.

In making the obviousness rejections, the Examiner contended that the previously submitted claims were not entitled to a priority date prior to the filing date of the present application. Specifically, the Examiner contended that that the parent application (07/148,959 filed 27 January 1988; the '959 application) only discloses an insertion of nucleic acid in a target sequence to make the reference sequence and does not disclose any other reference sequence. The Examiner also contended that the parent application does not disclose use of a heterologous reference sequence in which the target and reference sequences are amplified using their own primers. On the basis of this analysis, the Examiner has concluded that neither priority nor benefit of the early filing date will be given to these types of embodiments which have 1 September 1989 as their effective filing date. Applicants submit that the Examiner is in error in this analysis and conclusion as it relates to reference RNA sequence of claims 114-151 and the to the reference RNA sequence of claims 190-234. Applicants also submit that the Examiner is in error in this analysis and conclusion as it relates to the heterologous reference RNA sequence (i.e., a reference sequence which does not include target viral RNA sequence) of the previously submitted claims. Applicants note that reference RNA sequence of claims 152-189, e.g., a

minigene, does not appear *in haec verba* in the parent '959 application. However, Applicants submit that this reference RNA sequence is supported by the parent application in its disclosure that the target viral sequence and the reference RNA sequence are distinguishable by size.

Applicants submit that the disclosure of reference sequences is found in the '959 application. Specifically, Applicants note that the '959 application states at page 1, lines 20-24: "This invention adapts the Murakawa two primer amplification procedure to the identification and **quantification** of viral RNA ... More particularly such RNA is amplified simultaneously **with at least one other RNA sequence** ..." (emphasis added). Page 3 of the '959 application then describes the "at least one other RNA sequence." The first sequence that is described is a sequence that is unique to the T cell receptor. See page 3, lines 1-3 of the '959 application. This sequence is heterologous to the target viral RNA sequence. The second sequence that is described is a sequence that is ubiquitously present in all of the cells of the sample. See page 3, lines 12-13 of the '959 application. This sequence is also heterologous to the target viral RNA sequence. The fourth sequence that is described is "a reference RNA that can be amplified and detected by the same oligonucleotides used for authentic virus RNA sample." See page 3, lines 25-26 of the '959 application. This sequence is termed a "maxigene" which contains an insert into the target viral RNA sequence. See page 3, lines 27-28 of the '959 application. One example of such an insert is a multibase insert into the HIV-1 '3 ORF region. See page 3, lines 27-31 of the '959 application. Because the insert sequence is not a target viral RNA sequence, the "maxigene" comprises target viral RNA sequence and non-target viral RNA sequence. The amplified target viral RNA sequence and the amplified reference sequence are distinguishable by size. See page 4, lines 13-14. Reference sequences with a multibase insert or a multibase deletion are capable of being distinguished from the target viral RNA sequence by size. Thus, the '959 application describes several reference RNA sequences that can be used for identification and quantitation.

Applicants also submit that usage of a heterologous reference sequence in which the target sequence and the reference sequence are amplified by their own respective primer sets is found in the '959 application. Specifically, Applicants note that the '959 application describes the simultaneous amplification of the target viral sequence and the reference RNA sequence

using primer sets for each sequence. On page 2 of the '959 application, primer sets are disclosed for use in amplifying target viral RNA sequences from HIV-1 and HCMV. Primers for amplifying the T cell receptor beta chain and primers for amplifying beta-actin, two examples of heterologous reference sequences (or reference RNA sequences which do not include target viral RNA sequence) are disclosed at page 3 of the '959 application. With respect to the "maxigene," the '959 application discloses that it can be amplified using the same primers as the target viral RNA sequence. See page 3, lines 23-26 of the '959 application. Example I on pages 5-7 of the '959 application describes the simultaneous amplification of a target viral RNA sequence (HIV-1) and a heterologous reference RNA sequence (T cell receptor beta chain). These RNA sequences are amplified by their own respective primers sets. See page 5, line 29 - page 6, line 2 of the '959 application which discloses the addition of the HIV-1 primers and T cell receptor beta chain primers to the amplification mix. Figure 1 exemplifies the simultaneous amplification using primer sets for the HIV-1 target sequence and the T cell receptor beta chain, i.e., a heterologous reference sequence. Example II indicates that the procedure of Example I is repeated except that primers for beta-actin are used. Example III indicates that the procedure of Example I is repeated except that the maxigene is used. Thus, the '959 application describes the usage of a heterologous reference sequence in which the target and reference sequences are amplified each by their own primer sets.

In view of the above analysis of the disclosure in the '959 application, it is submitted that the '959 application fully describes the reference RNA sequence of claims 114-151, the reference RNA sequence of claims 152-189 and the reference sequence of claims 190-234, as well as the heterologous reference sequence set forth in the previously submitted claims. The '959 application also fully describes a heterologous reference sequence in which the target and reference sequences are each amplified by their own respective primer sets. Therefore, Applicants submit that this subject matter has priority and benefit to the '959 application. Thus, Applicants submit that the priority date for this subject matter is the filing date of the '959 application, i.e., 27 January 1988.

Furthermore, Applicants submit that additional priority for the claimed invention is found in application Serial No. 07/143,045, filed on 12 January 1988 (the '045 application). The '045

application describes the quantitation of viral RNA with HCMV as the example of the viral RNA. The viral RNA is quantified by including a fixed amount of an internal standard in the sample before amplification. The example of the internal standard is the target viral RNA sequence with a small insert. See page, 8, lines 8-18 of the '045 application. Because the internal standard contains a small insert, it contains a multibase insert. In addition, because the insert sequence is not a target viral RNA sequence, the internal standard comprises target viral RNA sequence and non-target viral RNA sequence. The amplified target viral RNA sequence and the amplified internal standard are distinguishable by size. The HCMV viral RNA of the '045 application is a second example of a viral RNA in addition to the HIV viral RNA of the '959 application. The quantitation of the HCMV viral RNA using a fixed amount of a reference RNA sequence that contains an insert as described in the '045 application is a second example of the quantitation of viral RNA in addition to the example of the quantitation of the HIV viral RNA of the '959 application.

In view of the above analysis of the disclosure in the '045 and '959 applications, it is submitted that the present application has priority to January 1988 for the reference RNA sequence of claims 114-151, the reference RNA sequence of claims 152-189 and the reference sequence of claims 190-234.

New Matter Rejection

The Examiner rejected all of the previously pending claims, i.e., claims 34, 35, 38, 39, 42-44 and 50-113 under 35 U.S.C. § 112, first paragraph for new matter. The Examiner contends that the specification as filed regarding a reference viral RNA sequence reveals that "only insertion of HIV-1 3'ORF (nef) sequence to result in a larger reference viral RNA sequence for usage in amplification mixtures is set forth in the entire original specification as filed on page 6, lines 15-29." The Examiner further contends that "[N]o other viral RNA insertion for reference preparation has been disclosed as originally filed." Thus, the Examiner contends that these claims contain new matter because they contain "limitations to non-target RNA viral sequences which are 'generic' which are thus not disclosed as filed." In addition, the Examiner contends that the separate and sequential probe removal and hybridization in claims 50

and 53 is not disclosed in the application as filed and thus is new matter. Applicants submit that the Examiner is in error in this rejection.

First, although Applicants believe that reference sequence (i) of the previously presented claims is not new matter because the specification fully describes generic reference RNA sequence (i) at page 4, lines 1-5 and at page 4, line10 - page 6, line 14 by describing several control, i.e., reference, RNA sequences that are not the target viral RNA sequence and that original claims 18 and 19 disclose reference RNA sequences that do not include target viral RNA sequence, they have nevertheless deleted this reference RNA sequence from the claims.

Second with respect to the reference RNA sequence of claims 114-151, i.e., a reference RNA sequence that consists of the target viral RNA sequence with a multibase insert into a site within the target viral RNA sequence (which corresponds to reference sequence (ii) of the previously presented claims), Applicants note that the specification at page 6, lines 19-21 states: "Such a reference sequence may be a ... 'maxigene' formed by a multi-base pair insert into ... a unique site." This reference RNA sequence is one that "can be amplified and detected by the same oligonucleotides used for authentic virus RNA samples." See page 6, lines 16-18. This description in the specification is a description of a generic maxigene. The specification provides two examples. The first example, which was noted by the Examiner, is an insert into the HIV-1 3' ORF (nef) gene. See page 6, lines 22-25. The second example, which was not noted by the Examiner, is an insert into the IE1 gene of HCMV. See page 7, lines 10-15. Also, original claim 18 states that the reference sequence is one which includes substantially more nucleotides than the target viral RNA sequence. In addition, original claim 19, which is dependent on claim 18 and further defines the reference sequences claimed in claim 18, states that the reference sequence is a sequence including the target viral RNA sequence and constructed by a multi-base insertion into a site in the target viral RNA sequence. Original claims 26-28 and 30 are directed to the quantitation of a target viral RNA sequence using the reference sequences of claim 18. In view of the disclosure in the specification and in the claims as originally filed, it is submitted that the present application fully describes the reference RNA sequence of claims 114-151. Thus, Applicants submit that reference RNA sequence of claims 114-151 is not new matter.

Third, and although the Examiner did not contend that the reference RNA sequence of claims 152-189, i.e., a reference RNA sequence that consists of the target viral RNA sequence with a multibase deletion from a site within the target viral RNA sequence (which corresponds to reference sequence (iii) of the previously presented claims), was new matter, Applicants note that the specification at page 6, lines 19-21 states: "Such a reference sequence may be a 'minigene' formed by a ... deletion of at lest about 20 nucleotides from a unique site." This reference RNA sequence is one that "can be amplified and detected by the same oligonucleotides used for authentic virus RNA samples." See page 6, lines 16-18. This description in the specification is a description of a generic minigene. In view of the disclosure in the specification, it is submitted that the present application fully describes the reference RNA sequence of claims 152-189. Thus, Applicants submit that reference RNA sequence of claims 152-189 is not new matter.

Fourth, with respect to reference RNA sequence of claims 190-234, i.e., a reference RNA sequence that comprises a sequence present in the target viral RNA sequence and a sequence that is not present in the target viral RNA sequence (which corresponds to reference sequence (iv) of the previously presented claims),, Applicants note that the specification at page 6, lines 19-21 states: "Such a reference sequence may be a ..."'maxigene' formed by a multi-base pair insert into ... a unique site." This reference RNA sequence is one that "can be amplified and detected by the same oligonucleotides used for authentic virus RNA samples." See page 6, lines 16-18. This description in the specification is a description of a generic maxigene. The specification provides two examples. The first example, which was noted by the Examiner, is an insert into the HIV-1 3' ORF (nef) gene. See page 6, lines 22-25. The second example, which was not noted by the Examiner, is an insert into the IE1 gene of HCMV. See page 7, lines 10-15. Also, original claim 18 states that the reference sequence is one which includes substantially more nucleotides than the target viral RNA sequence. In addition, original claim 19, which is dependent on claim 18 and further defines the reference sequences claimed in claim 18, states that the reference sequence is a sequence including the target viral RNA sequence and constructed by a multi-base insertion into a site in the target viral RNA sequence. Original claims 26-28 and 30 are directed to the quantitation of a target viral RNA sequence using the

reference sequences of claim 18. Because the insert is non-target viral RNA sequence, Applicants submit that the specification describes a genus of reference sequences which comprises target viral RNA sequence and non-target viral RNA sequence, as well as describing a genus of reference sequences that consists of a linear arrangement of a sequence present in the target viral RNA sequence, a sequence not present in the target viral RNA sequence and a sequence present in the target viral RNA sequence. In view of the disclosure in the specification and in the claims as originally filed, it is submitted that the present application fully describes the reference RNA sequence of claims 190-234. Thus, Applicants submit that reference RNA sequence of claims 190-234 is not new matter.

Fifth, although Applicants believe that the separate and sequential probe removal and hybridization language in claims 50 and 53 is not new matter because the application clearly describes this aspect of the claims at, for example, page 7, lines 25-27 (describing the use of labeled probes complementary to each of the amplified sequences and that these probes are used sequentially), page 12, lines 9-14 and 25-29 (stating that the probes are used separately and sequentially and that the first probe is removed before the second probe is used) and original claim 18 (setting forth this feature), they have deleted this aspect from the language of the newly submitted claims.

In view of the amendments to the claims and the above remarks, it is submitted that each of the claimed reference RNA sequences are fully described in the specification and claims as filed and thus do not constitute new matter. Withdrawal of this rejection is requested.

Obviousness Rejections

The Examiner entered two rejections of the claims under 35 U.S.C. § 103(a). In the first of these rejections the Examiner has rejected claims 34, 35, 38, 39, 44, 56, 59-63, 66, 67, 70, 71, 74-77, 80, 83, 86-89, 92, 95, 98, 99, 102, 104, 105-108 and 111 under 35 U.S.C. § 103(a) as being obvious over Chelly et al. (Nature 333:858, 1988) in view of Mullis et al. (US 4,683,195). The Examiner contends that Chelly et al. discloses the simultaneous amplification of a dystrophin target sequence and an aldolase A sequence. The aldolase A sequence is smaller than the dystrophin target sequence. The Examiner contends that Chelly et al. describes determining

quantitative amounts of the dystrophin target sequence. Thus, the Examiner concludes that reference sequences (i) and (iii) as set forth in the previously submitted claims are described in Chelly et al. Mullis et al. is cited to show that amplification of a viral sequence and to provide a suggestion and motivation for amplifying a viral target sequence with a heterologous sequence. Thus, the Examiner asserts that it would have been obvious for a skilled artisan to apply the amplification techniques of Chelly et al. to any source of nucleic acid, including viral sources, as motivated and suggested by Mullis et al. to result in the practice of the instant invention producing the improvements in quantitation in PCR as taught by Chelly et al. Applicants submit that the Examiner is in error in this rejection.

Chelly et al. describes the simultaneous amplification of a heterologous reference sequence and a target sequence. The heterologous reference sequence is smaller than the target sequence so that the amplification products can be distinguished by size. Chelly et al. does not describe a reference RNA sequence that includes target viral RNA sequence. That is, there is no disclosure in Chelly et al. of a reference RNA sequence that is (a) a reference RNA sequence that consists of the selected target viral RNA sequence with a multibase insert into a site within the selected target viral RNA sequence, (b) a reference RNA sequence that consists of the selected target viral RNA sequence with a multibase deletion from a site within the selected target viral RNA sequence or (c) a reference RNA sequence that comprises a sequence present in the selected target viral RNA sequence and a sequence not present in the selected target viral RNA sequence. Thus, Chelly et al. does not describe or suggest the reference RNA sequences of the newly submitted claims. Consequently, this element of the amended claims is missing from Chelly et al.

Chelly et al. describes a process in which a sample is subjected to the simultaneous amplification of a target mRNA, specifically dystrophin mRNA, and a standard mRNA, specifically aldolase A mRNA. Both the dystrophin mRNA and aldolase A mRNA were present in the sample, and neither was separately added to the sample. Thus, a known quantity of the control sequence was not used by Chelly et al. That is, Chelly et al. does not disclose or suggest the addition or use of a known quantity of a reference RNA sequence. Thus, this element of the claims is also missing from Chelly et al.

Chelly et al. also does not describe a method for the precise quantitation of a target RNA sequence, but merely describes a method for the crude estimation of the relative amount of the target dystrophin mRNA. Chelly et al. explains that aldolase A mRNA was used to check efficiency of the amplification reaction. See page 858, left column ("To check the efficiency of the procedure, we simultaneously co-reverse transcribed and co-amplified another transcript as an internal standard in the same test tube."). The calculations to determine the relative amount of the target dystrophin mRNA in the sample (relative to total mRNA) were based on the ratio of target to standard and the estimated amount of standard mRNA in certain specific tissues. See page 859, left column ("From this value we deduced the amount of dystrophin mRNA relative to total mRNA (Table 1)."). In this deduction, Chelly et al. provides, at best, an estimate that the amount of aldolase A mRNA ranges from about "0.1-0.5% of total mRNA in skeletal muscle, and at least ten times less in other tissues." See page 859, left column. The title of Table 1 ("Estimation ...), as well as the abstract ("quantitative estimate"), confirms that Chelly et al. only estimated the amount of dystrophin mRNA in the sample. Applicants submit that a quantitative estimate is not quantitation and no quantitative amount is provided by Chelly et al. Chelly et al. also states that the absolute value of dystrophin mRNA can not be determined directly but a relative figure can be deduced. See legend to Figure 3 under "Methods." Chelly et al. requires the use of ratios to measure expression of the target dystrophin mRNA because the standard, aldolase A, is not used in a known initial amount, as required by the present invention. It is the use of a known quantity of the reference RNA sequence in the claimed method, an element missing from Chelly et al., which enables the quantitation of target viral RNA, because the initial amount of the reference sequence is known.

From the above analysis of Chelly et al., it is apparent that Chelly et al.'s experimental objective was to provide a relative estimate of the amount mRNA present in a sample, such that the estimate was, at best, a comparative estimate. There is no attempt to use PCR to quantitate actual amounts of target. This objective differs from Applicants' objective which was to use PCR to quantitate a target viral RNA. Because Chelly et al.'s objective differs from Applicants' objective, it is evident that Chelly et al. could not motivate or suggest the claimed invention even if the critical elements of the present invention (discussed above) were disclosed therein.

Mullis et al. does not describe or suggest the quantitation of a target viral RNA in a sample, does not describe or suggest the use of an internal control in a quantitation method and does not describe or suggest the use of a known quantity of a reference RNA sequence as the internal control in a quantitation method. Thus, the combination of Mullis et al. with Chelly et al. does not describe or suggest the quantitation of a target viral RNA in a sample and does not describe or suggest the use of a known quantity of a reference RNA sequence as the internal control in a quantitation method. That is, the elements missing from Chelly et al. are not provided by Mullis et al.

In addition, the combination of Mullis et al. with Chelly et al. does not suggest using an internal control in a quantitation method in which the internal control is a reference sequence that includes target viral RNA sequence, i.e., a reference RNA sequence that is (a) a reference RNA sequence that consists of the selected target viral RNA sequence with a multibase insert into a site within the selected target viral RNA sequence, (b) a reference RNA sequence that consists of the selected target viral RNA sequence with a multibase deletion from a site within the selected target viral RNA sequence or (c) a reference RNA sequence that comprises a sequence present in the selected target viral RNA sequence and a sequence not present in the selected target viral RNA sequence.

Because reference RNA sequence of claims 114-151 (i.e., the target sequence with a multibase insert) has a priority date prior to the publication date of Chelly et al., Chelly et al. is not available as prior art with respect to this aspect of the claimed invention. Thus, the claimed invention with this reference RNA sequence is not obvious from the cited prior art.

Because reference RNA sequence of claims 152-189 (i.e., the target sequence with a multibase deletion) has a priority date prior to the publication date of Chelly et al., Chelly et al. is not available as prior art with respect to this aspect of the claimed invention. Thus, the claimed invention with this reference RNA sequence is not obvious from the cited prior art.

Because reference RNA sequence of claims 190-234 (i.e., a target sequence and a non-target sequence) has a priority date prior to the publication date of Chelly et al., Chelly et al. is not available as prior art with respect to this aspect of the claimed invention. Thus, the claimed invention with this reference RNA sequence is not obvious from the cited prior art.

Even assuming that the present claims are not entitled to the priority dates of the parent '045 and '959 applications, Applicants submit that the present claims are patentable over Chelly et al. in view of Mullis et al. Specifically, because Chelly et al. in combination with Mullis et al. does not disclose or suggest the use of an internal control that includes target viral RNA sequence, the claimed invention with the reference RNA sequences of claims 114-151, 152-189 and 190-234 of the newly submitted claims is not obvious from the cited prior art.

Even assuming that the present claims are not entitled to the priority dates of the parent '045 and '959 applications, Applicants submit that the present claims are patentable over Chelly et al. in view of Mullis et al. Specifically, because Chelly et al. in combination with Mullis et al. does not disclose or suggest the other elements of the claimed invention, i.e., the quantitation of a target viral RNA sequence and the use of a known quantity of the internal control, the claimed invention with respect to the reference RNA sequences of claims 114-151, 152-189 and 190-234 of the newly submitted claims is not obvious over the cited prior art.

Even assuming that the present claims are not entitled to the priority dates of the parent '045 and '959 applications, Applicants submit that the present claims are patentable over Chelly et al. in view of Mullis et al. Specifically, because Chelly et al. does not describe three features of the claimed invention, i.e., the specifically claimed reference RNA sequences, the use of a known quantity of the claimed reference RNA sequences and the quantitation of a target viral RNA sequence and because Mullis et al. does not provide these missing features, the presently claimed invention is not obvious from the combination of Chelly et al. and Mullis et al.

In view of the amendments to the claims and the above remarks, it is submitted that the claims are not obvious over the combination of Chelly et al. in view of Mullis et al. Withdrawal of this rejection is requested.

In the second obviousness rejection, the Examiner has rejected claims 34, 35, 38, 39, 42-44, 56, 59-63, 66, 67, 70-72, 74-77, 80, 83, 84, 86-89, 92, 95, 98, 99, 102, 104, 105-108 and 111 under 35 U.S.C. § 103(a) as being obvious over Chelly et al. (Nature 333:858, 1988) in view of Mullis et al. (US 4,683,195) and further in view of Sninsky et al. (US 5,176,995). Chelly et al. and Mullis et al. were cited as in the previous rejection. Sninsky et al. is cited to show the amplification of HIV. Because Sninsky et al. does not add anything to the combination of Chelly

et al. and Mullis et al. with respect to the claimed features of the invention and the elements missing from Chelly et al. and Mullis et al., i.e., quantitation of viral RNA, use of the claimed reference RNA sequences and use of a known quantity of the reference RNA sequence, its combination with these references does not render the claims obvious for the same reasons as detailed above with respect to the combination of Chelly et al. with Mullis et al.

In view of the amendments to the claims and the above remarks, it is submitted that the claims are not obvious over the combination of Chelly et al. in view of Mullis et al. and further in view of Sninsky et al. Withdrawal of this rejection is requested.

Comments Concerning References Listed in Information Disclosure Statements

In reviewing the Information Disclosure Statement filed on 4 June 2004, Applicants discovered an error in the listing of one of the patents. U.S. Patent No. 5,110,810 was listed instead of U.S. Patent No. 5,110,802. This error is being corrected by the filing of a Supplemental Information Disclosure Statement concurrently herewith. In addition to the listing of the '802 patent, the Supplemental Information Disclosure Statement also lists two additional Rossi patents, i.e., U.S. Patent Nos. 5,783,391 and 5,869,249. These two patents are related to U.S. Patent No. 5,622,820 identified on the previously submitted Information Disclosure Statement.

Cantin et al., U 5,110,802 issued from U.S. patent application Serial No. 07/073,189 filed on 14 July 1987. Cantin et al. discloses the simultaneous PCR amplification of a target viral RNA sequence and a fixed concentration of a reference sequence with an insert between the primer sites of the target sequence (column 4, lines 45-53). Cantin et al. further discloses that the initial amount of reference sequence template remains constant thereby enabling one to determine the ratio of amplification of the reference sequence template versus the sample template before and after treatment to determine the effect of treatment on viral RNA synthesis (column 4, lines 57-60 and lines 44-45). The subject matter of the claims of the present application, which is not claimed in Cantin et al, was the invention of Murakawa, Wallace, Zaia and Rossi, the inventors of the present application, and not the invention of Cantin. See

Declaration of John J. Rossi, Ph.D. that was filed as Exhibit MX 1021 in Interference No. 105,055 (copy attached to the Amendment filed on 4 June 2004).

Rossi, US 5,622,820, issued from U.S. application Serial No. 08/334,398 filed on November 3, 1994 as a continuation application of U.S. application Serial No. 07/180,740 filed on April 12, 1988. The latter application was a continuation-in-part of U.S. application Serial No. 07/165,915 filed on March 10, 1988. Thus, the earliest effective filing date for Rossi is either April 12, 1988 or March 10, 1988, both of which are after the filing dates of the parent '045 and '959 applications to which Applicants have shown that they are entitled to priority for the presently claimed invention. In view of this priority, Rossi '820 is not prior art. These comments also apply to Rossi, US 5,783,391, and Rossi, US 5,869,249.

Even assuming that the present claims are not entitled to the priority dates of the parent '045 and '959 applications, Applicants submit that the present claims are patentable over Rossi '820. Specifically, Rossi '820 discloses an internal control for use in determining whether the PCR reaction was successful (see column 3, lines 43-46) for the detection of false positives (see claims 6-16). The internal control is the target sequence with a 21 base insert into the target region. See Example 1. A simultaneous amplification of a plasmid containing a target sequence and a plasmid containing the target sequence with the internal control was performed and the results analyzed following a transcription reaction. See Example 2 and 3. The amplification of a sample containing a target viral RNA sequence is disclosed in Example 4. According to Example 4, the target viral RNA sequence was amplified for 15 rounds. A portion of the reaction mixture was removed and mixed with 50 ng of the internal control (target viral RNA sequence with a multibase insert). This mixture was then simultaneously amplified for 10 rounds and then analyzed for the presence of the amplified products.

Although Rossi '820 discloses a reference RNA sequence that includes target viral RNA sequence, Rossi '820 does not disclose the quantitation of a target viral RNA. Rossi '820 also does not disclose the addition of a known quantity of the reference RNA sequence to a sample containing a target viral RNA sequence followed by the simultaneous amplification of the target viral RNA sequence and the reference RNA sequence. Finally, Rossi '820 does disclose measuring the amount of the amplified products and determining the amount of target viral RNA

originally present in the sample from the amounts of the amplified target viral RNA sequence and the amplified reference RNA sequence. Thus, Rossi '820 does not teach or suggest the presently claimed invention. Furthermore, there is no suggestion or motivation in the prior cited by the Examiner (i.e., Chelly et al., Mullis et al. and Sninsky et al.) that would lead a skilled artisan to combine Rossi '820 with the cited prior art. Thus, Applicants submit that the presently claimed invention is patentable over Rossi '820 and the cited prior art. These comments also apply to Rossi, US 5,783,391, and Rossi, US 5,869,249.

Murakawa et al. (*DNA* 7:287-295, 1988) discloses an internal control for use in determining whether the PCR reaction was successful. See page 293, right column. The internal control is the target sequence with a 21 base insert into the target region. See page 292, right column. A simultaneous amplification of a plasmid containing a target sequence and a plasmid containing the target sequence with the internal control was performed and the results analyzed following a transcription reaction. See page 292, right column and legend to Figure 7. The amplification of a sample containing a target viral RNA sequence is disclosed in the legend to Figure 8. According to this description, the target viral RNA sequence was amplified for 15 rounds. A portion of the reaction mixture was removed and mixed with 5 ng of the internal control (target viral RNA sequence with a multibase insert). This mixture was then simultaneously amplified for 10 rounds and then analyzed for the presence of the amplified products. The publication date of Murakawa et al. is subsequent to the filing dates of the parent '045 and '959 applications to which Applicants have shown that they are entitled to priority for the presently claimed invention. In view of this priority, Murakawa et al. is not prior art.

Even assuming that the present claims are not entitled to the priority dates of the parent '045 and '959 applications, Applicants submit that the present claims are patentable over Murakawa et al. Although Murakawa et al. discloses a reference RNA sequence that includes target viral RNA sequence, Murakawa et al. does not disclose the quantitation of a target viral RNA. Murakawa et al. also does not disclose the addition of a known quantity of the reference RNA sequence to a sample containing a target viral RNA sequence followed by the simultaneous amplification of the target viral RNA sequence and the reference RNA sequence. Finally, Murakawa et al. does disclose measuring the amount of the amplified products and determining

the amount of target viral RNA originally present in the sample from the amounts of the amplified target viral RNA sequence and the amplified reference RNA sequence. Thus, Murakawa et al. does not teach or suggest the presently claimed invention. Furthermore, there is no suggestion or motivation in the prior cited by the Examiner (i.e., Chelly et al., Mullis et al. and Sninsky et al.) that would lead a skilled artisan to combine Murakawa et al. with the cited prior art. Thus, Applicants submit that the presently claimed invention is patentable over Murakawa et al. and the cited prior art.

Zaia and Rossi (1989a) (*Transfusion Medicine Reviews* **3**(1), Suppl. 1:27-30, 1989) and Zaia and Rossi (1989b) (*TestTrends* **3**(1):4-5, 1989) disclose the simultaneous amplification of a target viral RNA sequence that may be present in a sample and a reference RNA sequence in which the reference RNA sequence differs in length and in which a known quantity of the reference RNA sequence is used for the quantitation of the target viral RNA sequence. The publication date of both of the Zaia and Rossi references is subsequent to the filing dates of the parent '045 and '959 applications to which Applicants have shown that they are entitled to priority for the presently claimed invention. In view of this priority, Zaia and Rossi (1989a) and Zaia and Rossi (1989b) are not prior art.

Even assuming that the present claims are not entitled to the priority dates of the parent '045 and '959 applications, Applicants submit that the present claims are patentable over Zaia and Rossi (1989a) and Zaia and Rossi (1989b) because the quantitation subject matter disclosed in these references, which were published less than one year before the filing date of the '450 application, was the invention of Murakawa, Wallace, Zaia and Rossi. See Declaration of John J. Rossi attached hereto.

Wang et al. (US 5,219,727) and Wang et al. (US5,476,774) disclose the quantitation of a target nucleic acid by the simultaneous amplification of a target nucleic acid that may be present in a sample and a predetermined amount of a standard nucleic acid that was added to the sample. The target and sample bind the same primers, and the amplified products are distinguishable by size or probes. The earliest filing date of both Wang et al. patents is subsequent to the filing dates of the parent '045 and '959 applications to which Applicants have shown that they are

entitled to priority for the presently claimed invention. In view of this priority, Wang et al. '727 and Wang et al., '774 are not prior art.

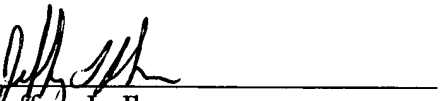
Even assuming that the present claims are not entitled to the priority dates of the parent '045 and '959 applications, Applicants submit that the present claims are patentable over Wang et al. '727 and Wang et al. '774 because Applicants invented the claimed subject matter of the present application prior to the earliest filing date of these patents, i.e., before 21 August 1989. This fact is evidenced by a Declaration Under 37 C.F.R. § 1.131(a) that is currently being executed by the inventors. A copy of the unexecuted Declaration is attached hereto for the convenience of the Examiner. The executed Declaration will be submitted as soon as it is received.

Concluding Comments

In view of the above amendments and remarks, it is submitted that the claims are fully supported by the instant application and are patentable over the prior art of record. Reconsideration of this application and early notice of allowance is requested. The Examiner is invited to telephone the undersigned if it will assist in expediting the prosecution and allowance of the instant application.

Respectfully submitted,

ROTHWELL, FIGG, ERNST & MANBECK, p.c.

By 

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Attachments: Unexecuted Declaration Under 37 C.F.R. § 1.131(a)
Declaration of John J. Rossi